The Diagnostic Value Of Soluble CD93 In Chinese Patients With Type 2 Diabetic Nephropathy

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Abstract

Objective: Our aim was to explore the diagnostic value of serum soluble cluster of differentiation 93 (sCD93) for diabetic nephropathy (DN) in chinese patients.

Methods: 130 patients with type 2 diabetes mellitus (T2DM) and 30 healthy individuals were enrolled. Enzyme-linked immunosorbent assay (ELISA) was used to determine the serum sCD93 concentration. And renal function and blood lipid-related index in serum or urine were detected using the routine assays. A receiver operating characteristic (ROC) curve was exployed to evaluate the diagnostic efficacy of integrated indicators.

Results: The eGFR, ACR ($P < 0.05$) and the prevalence of DN ($P < 0.05$) were significantly different between the H-sCD93 group and the L-sCD93 group. The serum sCD93 concentration was correlated with the ACR (r = 0.191, $P = 0.029$) and eGFR (r = -0.509, $P = 0.000$). In all albuminuria subgroups, the serum sCD93 concentration increased with the increase in the ACR ($P < 0.05$). Pairwise comparisons between subgroups indicated that the serum sCD93 concentrations from the N-ACR and M-ACR groups were lower than that in the L-ACR group ($P < 0.05$), however there was no significant difference between the N-ACR and M-ACR groups ($P > 0.05$). The levels of serum sCD93, NGAL and Cr in DN group were significantly higher than those in DM group ($P<0.05$). On the basis of the ROC curve, the best AUC area value was 0.942 from sCD93+NGAL+Cr triple test.

Conclusion: sCD93 was a independent predictor for DN in chinese patients, and integration analysis of sCD93, NGAL and Cr has better diagnostic efficacy.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by chronic hyperglycemia, which is caused by defects in insulin secretion and/or action. Among them, type 2 diabetes mellitus (T2DM) accounts for approximately 90% of all DM cases. The produced hyperglycemia can disrupt hemodynamics and metabolic homeostasis, causing patients to have a variety of serious complications[1-2]. Diabetic nephropathy (DN) is a key microvascular complication of DM, which is defined as increased urinary albumin excretion with no other identifiable clinical manifestations. DN is a common cause of end-stage renal failure and the main reason for the high risk of death among DM patients[3].

The classic markers of DN include the albumin-to-creatinine ratio (ACR) and estimated glomerular filtration rate (eGFR)[4]. However, albuminuria and reduced eGFR in DM patients are not only due to DN, and patients with early DN may not have albuminuria or reduced eGFR. Therefore, the 2 diagnostic markers have certain application limitations for the detection of DN, and thus, there is an urgent need to introduce new, noninvasive biomarkers to provide more available clinical evidence[5]. Currently, promising biomarkers include urinary neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), serum interleukin-18 (IL-18), and soluble cluster of differentiation 93 (sCD93)[6]. These new biomarkers are related to glomerular dysfunction, renal tubular dysfunction, oxidative stress, and inflammation. However, they have not been added to the established diagnostic items for DN, and more research data are needed to clarify their clinical value[7].

CD93 is a type 1 transmembrane protein composed of a C-type carbohydrate recognition domain (CRD), 5 epidermal growth factor (EGF)-like domains and a transmembrane domain (TMD)[8], whcih is mainly expressed

in monocytes and endothelial cells. Under inflammatory conditions, the extracellular portion of CD93 can be secreted as the sCD93[9]. Currently, sCD93 concentrations have shown to be associated with various inflammatory diseases, such as cerebral ischemia, coronary artery disease, systemic sclerosis, asthma and nasopharyngeal carcinoma[10-13]. DM is also an inflammatory disease that can lead to a series of chronic vascular complications. Studies have found that the concentration of serum sCD93 is higher in DN patients, and this concentration is not directly affected by glucose metabolism factors but is more regulated by inflammatory conditions in the internal environment. Therefore, sCD93 may be served as a new biological indicator of DN[14].

In this study, firstly, the relationships between serum sCD93 or traditional indicators of DN (eGFR and ACR) and the prevalence of DN were investigated. Secondly, the correlation between sCD93 and the degree of microalbuminuria in T2DM patients was analyzed. Finally, the diagnostic efficacy of combining sCD93, traditional markers and inflammatory indicators for DN was evaluated, so as to improve the diagnosis, treatment and prognosis of DN thereby preventing the occurrence of DM complications.

1. Subjects And Methods

1.1 Research subjects

A total of 130 T2DM patients (85 males and 45 females aged 35–90 (62.27 ± 11.26) years old) admitted to the Affiliated Hospital of North Sichuan Medical College between August 2020 and March 2021 were enrolled. The inclusion criteria were as follows: 1) patients who were ≥ 20 years of age and 2) patients who met the diagnostic criteria for T2DM released by the American Diabetes Association (ADA) in 2015. The exclusion criteria were as follows: 1) patients with concomitant malignant tumors; 2) patients with acute and chronic infectious diseases, such as rheumatoid arthritis and lupus; 3) patients using steroidal and nonsteroidal anti-inflammatory drugs; 4) patients with abnormal liver function; 5) patients with acute vascular diseases, such as acute myocardial infarction and stroke; and 6) patients with hypertension and other known diseases that can cause albuminuria: a) various types of nephritis or urinary tract obstruction; b) potassium-sparing diuretics and antacids; c) urinary tract infection (patients could be enrolled in the study only after normal urine for 2 weeks; and d) renal vascular disease. Based on the median serum sCD93 concentration, the study subjects were divided into a low serum sCD93 group (L-sCD93 group, n = 65) and a high serum sCD93 group (H-sCD93 group, n = 65). According to the ADA (2007) recommend (use the ACR in random urine specimens to diagnose microalbuminuria), subjects were divided into 3 subgroups based on their ACRs[15], i.e., 34 patients in the normal group (ACR < 30 μg/mg; N-ACR group), 33 patients in the microalbuminuria group (30μg/mg ≤ ACR ≤ 300 μg/mg; M-ACR group), 63 patients in the massive albuminuria group (ACR > 300 μg/mg; L-ACR group), and 30 healthy individuals in the control group (NC group). Based on their urine albumin-to-creatinine ratio (UACRs) and eGFRs, the study subjects were divided into 2 subgroups[16], i.e., 23 patients in the simple T2DM group (DM group; ACR < 30 mg/g, eGFR ≥ 90 $mL{\cdot}min^{-1}{\cdot}1.73\ m^{-2}$) and 107 patients in the DN group (clinical DN patients with ACR > 300 mg/g or eGFR < 60 $mL{\cdot}min^{-1}{\cdot}1.73\ m^{-2}$ and early DN patients with 30 mg/g ≤ ACR < 300 mg/g and eGFR ≥ 60 $mL{\cdot}min^{-1}{\cdot}1.73m^{-2}$ or ACR < 30 mg/g but 60 $mL{\cdot}min^{-1}{\cdot}1.73\ m^{-2}$ ≤ eGFR < 90 $mL{\cdot}min^{-1}{\cdot}1.73m^{-2}$).

1.2 Research methods

Sex, age, course of DM, height, weight, medication use, abnormal blood lipids, and macrovascular and microvascular complications were collected from the patient's medical records (clinical records). Serum (2 mL) and random urine (2 mL) samples were collected from the subjects and stored at -80°C. Serum NGAL, fasting blood glucose (FBG), total cholesterol (TC), and high-density lipoprotein (HDL) concentrations were determined using a Cobas 6000 (Roche Biochemical Analyzer) and supporting reagents; low-density lipoprotein cholesterol (LDL) concentration was calculated using the Friedewald formula, the concentrations of biochemical and immune indicators, such as triglycerides (TG), serum creatinine (Cr, sarcosine oxidase method), serum total protein (TP), serum albumin (Alb), and alanine aminotransferase (ALT), were determined, and the eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, which includes serum Cr concentration, sex and age[17]. UACR in a random urine sample = urinary Alb / urinary Cr. The serum sCD93 concentration was determined using an enzyme-linked immunosorbent assay (ELISA). The ELISA kit used in this study was purchased from Invitrogen (USA), and the source was reliable. Double-antibody sandwich ELISA had the advantages of high sensitivity and high specificity. Serum sCD93 concentrations were determined strictly following the instructions provided with the kit. In the experimental procedure, negative and positive controls were included with each test, and the data were obtained from 3 replicate tests. All methods were carried out in accordance with relevant guidelines and regulations, all experimental protocols were approved by the ethics committee of the Affiliated Hospital of North Sichuan Medical College and informed consent was obtained from all subjects and their legal guardian(s).

1.3 Statistical analysis

Statistical analysis was performed using SPSS 25.0, and $P < 0.05$ was considered statistically significant. Measurement data were expressed as the mean ± standard deviation (SD), and categorical data were expressed as numbers (%). To assess the differences between groups, measurement data were evaluated using the t test or the Kruskal-Wallis H test, and the categorical variables were evaluated using the x^2 test or Fisher's exact test. Correlations between 2 continuous variables were assessed; the results were expressed using the Pearson correlation coefficient (r). Multivariate stepwise regression analysis was used to investigate the linear dependence between the dependent variable and multiple independent variables. Logistic regression analysis was used to evaluate the relative risk of DN in the L-sCD9 and H-sCD93 groups. A receiver operating characteristic (ROC) curve was used to analyze diagnostic efficacy for type 2 DN when using serum sCD93, Cr and NGAL alone or in various combinations.

2. Results

2.1 Baseline characteristics of study subjects

The baseline characteristics of the DM patients were investigated, and the subjects were divided into a L-sCD93 group and H-sCD93 group based on the median serum sCD93 concentration. There were no significant differences in age, sex, body mass index (BMI), and the prevalence of cardiometabolic risk factors between the 2 groups ($P > 0.05$). The eGFR level was lower ($P = 0.000$), the ACR level was higher ($P = 0.000$), the serum NGAL level was higher ($P = 0.008$), and the Cr level was higher ($P = 0.000$) in the H-sCD93 group than in the L-sCD93 group (Table 1).

Table 1
Baseline characteristics of the study subjects (based on serum sCD93 concentration)

	Total (N = 130)	L-sCD93 (n = 65)	H-sCD93 (n = 65)	*P*
sCD93 (ng/mL)	317.53 ± 192.84	166.32 ± 52.54	468.74 ± 160.48	**0.000**
log(sCD93) (ng/mL)	2.45 ± 0.29	2.20 ± 0.15	2.70 ± 0.14	**0.000**
Age (years)	62.27 ± 11.26	61.74 ± 9.76	62.80 ± 12.64	0.593
Male rate (n, %)	85(65.4)	44(67.70)	41(63.1)	0.580
BMI (kg/m^2)	23.07 ± 3.45	22.91 ± 3.04	23.25 ± 3.84	0.599
Dyslipidemia rate (n, %)	27(20.8)	16(9.2)	11(16.9)	0.280
DM course (years)	10.86 ± 6.42	9.92 ± 5.47	11.80 ± 7.16	0.184
DM drugs				
Sulfonylurea hypoglycemic agents (n, %)	14(10.8)	10(15.4)	4(6.2)	0.155
Metformin (n,%)	47(36.2)	26(40.0)	21(32.3)	0.361
Dipeptidyl peptidase-4 inhibitor (n,%)	19(14.6)	9(13.8)	10(15.4)	0.804
Insulin (n,%)	108(83.1)	52(80)	56(86.2)	0.349
Other drugs				
ACE-I or ARB (n, %)	36(27.7)	17(26.2)	19(29.2)	0.695
Statins (n,%)	73(56.2)	34(52.3)	39(60.0)	0.377
Dapagliflozin tablets (n,%)	40(30.8)	24(36.9)	16(24.6)	0.128
Fibrates (n,%)	6(4.6)	4(6.2)	2(3.1)	0.680
FBG (mmol/L)	9.71 ± 3.12	9.75 ± 3.41	9.66 ± 2.83	0.871
Glycated hemoglobin A1c (HbA1c) (%)	9.84 ± 2.71	10.11 ± 2.81	9.57 ± 2.60	0.370
Cr (mmol/L)	171.00 ± 215.17	85.60 ± 80.50	255.40 ± 269.01	**0.000**
Serum TP (g/L)	67.40 ± 7.28	67.45 ± 7.31	67.36 ± 7.30	0.945
Serum Alb (g/L)	40.54 ± 17.00	40.65 ± 4.65	40.43 ± 23.69	0.109
ALT (U/L)	19.15 ± 11.46	18.06 ± 8.14	20.25 ± 14.01	0.918
TC (mmol/L))	4.69 ± 1.48	4.78 ± 1.45	4.60 ± 1.51	0.565

Data were expressed as the mean ± SD or number (%). The *P* value represents the difference between the groups as determined by the paired t test or Kruskal-Wallis H test for continuous variables or by the x^2 test or Fisher's exact test for categorical variables. Statistically significant values were indicated in bold ($P < 0.05$). DPP4 inhibitor, dipeptidyl peptidase-4 inhibitor; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; HbA1c, glycosylated hemoglobin; ALT, alanine aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACR, urinary albumin-creatinine ratio; eGFR, estimated glomerular filtration rate.

	Total (N = 130)	L-sCD93 (n = 65)	H-sCD93 (n = 65)	*P*
TG (mmol/L)	2.00 ± 1.69	2.01 ± 1.86	1.99 ± 1.51	0.328
HDL (mmol/L)	1.94 ± 8.59	2.72 ± 12.13	1.16 ± 0.39	0.242
LDL (mmol/L)	4.19 ± 20.34	2.51 ± 1.03	5.86 ± 28.76	0.289
Serum NGAL (μg/L)	390.72 ± 430.028	340.69 ± 286.56	611.51 ± 510.96	**0.008**
ACR (mg/g)	744.69 ± 1428.58	534.11 ± 1246.48	955.28 ± 1571.56	**0.000**
eGFR (mL/min/1.73 m^2)	75.48 ± 45.75	94.76 ± 37.96	56.20 ± 44.98	**0.000**

Data were expressed as the mean ± SD or number (%). The *P* value represents the difference between the groups as determined by the paired t test or Kruskal-Wallis H test for continuous variables or by the x^2 test or Fisher's exact test for categorical variables. Statistically significant values were indicated in bold ($P < 0.05$). DPP4 inhibitor, dipeptidyl peptidase-4 inhibitor; ACE-I, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BMI, body mass index; HbA1c, glycosylated hemoglobin; ALT, alanine aminotransferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ACR, urinary albumin-creatinine ratio; eGFR, estimated glomerular filtration rate.

2.2 Differences in the prevalence of DN between the L-sCD93 and H-sCD93 groups

The prevalence of macrovascular and microvascular complications in the study subjects was investigated (Table 2). There was no significant difference in the prevalence of macrovascular complications between the L-sCD93 and H-sCD93 groups. In terms of microvascular complications, there were more subjects with CKD stage ≥ 3 in the H-sCD93 group than in the L-sCD93 group (55.4% vs 18.5%). There were significantly more subjects in with ACR ≥ 300 mg/g in the H-sCD93 group than in the L-sCD93 group (67.7% vs. 29.2%).

Table 2
The prevalence of macrovascular and microvascular complications in the study subjects (based on the serum sCD93 concentration)

	Total	L-sCD93 group	H-sCD93 group	*P*
Macrovascular complications				
Cerebrovascular accident	21(16.2)	11(16.9)	10(15.4)	0.812
Coronary artery disease	30(23.1)	12(18.5)	18(27.7)	0.212
Peripheral artery disease	46(35.4)	26(40.0)	20(30.8)	0.271
≥ 2 macrovascular complications	14(10.8)	8(12.3)	6(9.2)	0.571
Microvascular complications				
Diabetic retinopathy	73(56.2)	37(56.9)	36(55.4)	0.860
Diabetic neuropathy	76(58.5)	31(47.7)	36(55.4)	0.286
DN				
≥ 2 microvascular complications	80(61.5)	39(60.0)	41(63.1)	0.718
CKD staging				
60 ≤ eGFR < 90 (stage 2)	35(26.9)	19(29.2)	16(24.6)	0.553
eGFR < 60 (≥ stage 3)	48(36.9)	12(18.5)	36(55.4)	**0.000**
Albuminuria				
30 ≤ ACR < 300 (microalbuminuria)	34(26.2)	18(27.7)	16(24.6)	0.690
ACR ≥ 300 (massive albuminuria)	63(48.5)	19(29.2)	44(67.7)	**0.000**

Data were expressed as numbers (%). *P* represents the difference between groups as determined by the x^2 test or Fisher's exact test. Statistically significant values were indicated in bold ($P < 0.05$). CKD, staging of chronic kidney disease; ACR, urinary albumin-creatinine ratio; eGFR, estimated glomerular filtration rate.

2.3 The relationships between serum sCD93 concentration and DN-related indicators

The correlations between serum sCD93 concentration and clinical parameters were evaluated (Table 3). Serum sCD93 concentration was positively correlated with ACR (r = 0.191, $P = 0.029$) and negatively correlated with eGFR (r = -0.509, $P = 0.000$). Serum sCD93 concentration was also significantly correlated with logarithmically transformed eGFR and ACR (Fig. 1A, B). Compared with the serum sCD93 concentration in patients with stage 1 CKD, the serum sCD93 concentration in patients with stage 2 and ≥ 3 CKD was significantly higher and showed a gradual increasing trend with CKD stage (Fig. 1C). Multivariate linear regression analysis was performed to verify the correlations between serum sCD93 and eGFR and between sCD93 and ACR (Table 4). Demographic parameters (such as age and sex), glucose metabolism parameters (such as BMI and HbA1c) and logarithmically transformed serum CD93 concentration were used as independent factors. After adjusting for confounding factors, the serum sCD93 concentration was a significant independent factor for the reduction in

eGFR (β = -78.552, standard error (SE) = 0.292, *P* = 0.000) and for the increase in ACR (β = 577.672, SE = 0.292, *P* = 0.042).

Table 3
Correlation between serum sCD93 and various clinical parameters

	Log (serum sCD93 concentration)	
	R	*P*
Age (years)	0.054	0.541
BMI (kg/m^2)	-0.021	0.812
FBG (mmol/L)	-0.041	0.645
HbA1c(%)	-0.086	0.329
Cr (mmol/L)	0.524	**0.000**
Serum NGAL (μg/L)	0.433	**0.000**
ACR (mg/g)	0.191	**0.029**
eGFR (mL/min/1.73 m^2)	-0.509	**0.000**

The data were expressed using the Pearson correlation coefficient (r). Statistically significant values were indicated in bold (*P* < 0.05). BMI, body mass index; HbA1c, glycosylated hemoglobin; ACR, urinary albumin-creatinine ratio; eGFR, estimated glomerular filtration rate.

Table 4
Correlations between serum sCD93 and eGFR and between serum sCD93 and ACR

	Univariate model				**Multivariate model**			
eGFR	Regression coefficient	SE	*P*	R^2	Regression coefficient	SE	*P*	Adjusted R^2
Age (years)	-0.453	11.301	0.207	0.012	-0.375	11.301	0.207	0.296
Sex(reference: female)	0.458	0.478	0.957	< 0.001	-2.988	0.478	0.669	
BMI (kg/m^2)	1.439	3.466	0.219	0.012	0.963	3.467	0.195	
HbA1c (%)	3.775	2.669	0.011	0.050	2.420	2.669	0.056	
Log (serum sCD93 concentration) (ng/mL)	-79.793	0.292	**0.000**	0.259	-78.552	0.292	**0.000**	
	Univariate model				Multivariate model			
ACR	Regression coefficient	SE	*P*	R^2	Regression coefficient	SE	*P*	Adjusted R^2
Age (years)	-12.316	11.260	0.272	0.009	-7.669	11.224	0.295	0.046
Sex(reference: female)	99.269	0.478	0.708	0.001	100.876	0.478	0.559	
BMI (kg/m^2)	-40.085	3.453	0.264	0.010	-18.847	3.475	0.427	
HbA1c (%)	-7.225	2.712	0.876	0.008	13.379	2.723	0.658	
Log (serum sCD93 concentration) (ng/mL)	937.988	0.292	**0.029**	0.037	577.672	0.292	**0.042**	

Data were expressed using the regression coefficient and SE. $P < 0.05$ is considered statistically significant. SE, standard error; BMI, body mass index; HbA1c, glycosylated hemoglobin.

2.4 The relationship between serum sCD93 concentration and the risk of DN

In the low sCD93 group compared with the high sCD93 group, the covariate-adjusted risk for either a developing stage ≥ 3 CKD or massive albuminuria and also, DN, a composite of both, were evaluated (Table 5). After adjusting for age, sex, BMI, and HbA1c and using the L-sCD93 group as the reference, the DM patients in the H-sCD93 group were more likely to have a decreased eGFR (adjusted odds ratio (OR) 5.848, 95% confidence interval (CI) 2.565–13.328, $P = 0.000$), more likely to develop massive albuminuria (adjusted OR 5.255, 95% CI 2.446–11.290, $P = 0.000$), and more likely to develop DN (adjusted OR 5.307, 95% CI 2.465–11.425, $P = 0.000$).

Table 5
The risk of renal complications in the H-sCD93 group

eGFR < 60 (CKD stage ≥ 3)			
	L-sCD93	H-sCD93	*P*
Model 1	1(reference)	5.483(2.476–12.139)	**0.003**
Model 2	1(reference)	5.453(2.457–12.102)	**0.000**
Model 3	1(reference)	5.848(2.565–13.328)	**0.000**
Massive albuminuria (ACR > 300)			
	L-sCD93	H-sCD93	*P*
Model 1	1(reference)	5.073(2.407–10.692)	**0.000**
Model 2	1(reference)	5.258(2.460-11.238)	**0.000**
Model 3	1(reference)	5.255(2.446–11.290)	**0.000**
DN (CKD stage > 3 or massive albuminuria)			
	L-sCD93	H-sCD93	*P*
Model 1	1(reference)	5.104(2.416–10.780)	**0.000**
Model 2	1(reference)	5.091(2.404–10.780)	**0.000**
Model 3	1(reference)	5.307(2.465–11.425)	**0.000**

Model 1, not adjusted; Model 2, adjusted for age and sex; Model 3, adjusted for age, sex, BMI and HbA1c. The data were expressed as ORs, and the L-sCD93 group is used as the reference. $P < 0.05$ is considered statistically significant. CKD, staging of chronic kidney disease; BMI, body mass index; HbA1c, glycosylated hemoglobin.

2.5 Comparison of serum sCD93 at different disease stages

The differences of sCD93 in different DN stages were evaluated (Table 6). The Goldman's Cecil Medicine.24th ed.2011staging method was combined with the ACR classification criteria recommended by ADA (2007) (i.e., determining the microalbuminuria in random urine specimens) for grouping. The general clinical data including age, HbA1c, Cr, FBG, serum NGAL and serum sCD93 concentrations were compared among the NC, N-ACR, M-ACR, and L-ACR groups, and the differences were statistically significant ($P < 0.05$). The general clinical data, Cr, serum NGAL and serum sCD93 concentrations were compared among the N-ACR, the M-ACR, and L-ACR groups, and the differences were statistically significant ($P < 0.05$). Pairwise comparisons of the 2 groups indicated that the Cr, serum NGAL, and serum sCD93 concentrations in the N-ACR and M-ACR groups were lower than those in the L-ACR group ($P < 0.05$); however, there were no statistically significant differences between the N-ACR and M-ACR groups ($P > 0.05$) (Table 6). The sCD93 concentration with respect to the microalbuminuria stage is shown in Fig. 1D.

Table 6
Comparison of different groups

	Entire DM group	NC group	N-ACR group	M-ACR group	L-ACR group
Number of cases (n)	130	30	34	33	63
Age (years) ♥	62.27 ± 11.26	45.70 ± 9.59	62.47 ± 9.28	63.73 ± 10.50	61.40 ± 12.61
FBG (mmol/L) ♥	9.71 ± 3.12	4.74 ± 0.41	9.10 ± 3.49	10.15 ± 3.25	9.81 ± 2.83
BMI (kg/m^2)	23.08 ± 3.45	22.41 ± 2.46	22.50 ± 2.71	23.58 ± 3.98	23.12 ± 3.54
HbA1c(%) ♥	9.84 ± 2.71	5.67 ± 0.43	9.67 ± 2.60	10.29 ± 2.72	9.69 ± 2.78
Cr (mmol/L) ♥♣	171.00 ± 215.17	57.58 ± 11.44	63.42 ± 19.31□	77.37 ± 30.92□	278.10 ± 270.12
Serum NGAL (μg/L) ♥♣	476.10 ± 434.45	48.79 ± 49.41	160.36 ± 136.76□	316.72 ± 203.12□	716.63 ± 495.31
Serum sCD93(ng/mL) ♥♣	317.53 ± 192.84	110.51 ± 65.90	196.92 ± 81.08□	270.39 ± 153.55□	407.310 ± 210.61

Data were expressed as the mean ± SD or number (%). For data without homogeneity of variance and without a normal distribution, and the nonparametric Kruskal-Wallis test was used; for data with a normal distribution and homogeneity of variance, univariate analysis of variance (ANOVA) and pairwise comparisons were used. Comparison among 4 groups, ♥ $P < 0.05$; comparison among 3 groups, ♣ $P < 0.05$; compared with the L-ACR group, □ $P < 0.05$. BMI, body mass index; HbA1c, glycosylated hemoglobin.

2.6 Comparison of renal function indicators between the DM and DN groups

The comparisons indicated that the ACR and serum Cr, serum NGAL, and serum sCD93 concentrations in the DN group were higher than those in the DM group ($P = 0.000$). The eGFR in the DN group was lower than that in the DM group ($P = 0.000$) (Table 7).

Table 7
Comparisons of renal function indicators between the DM and DN groups

	DM group	DN group	*P*
Number of patients	23	107	
Cr (mmol/L)	52.72 ± 9.60	196.43 ± 229.42	0.000
Serum NGAL (μg/L)	172.56 ± 131.79	541.35 ± 449.23	0.000
Serum sCD93 (ng/mL)	183.78 ± 64.57	346.28 ± 199.19	0.000
ACR (mg/g)	12.50 ± 7.26	902.08 ± 1530.47	0.000
eGFR (mL/min/1.73 m^2)	128.59 ± 26.75	64.07 ± 40.69	0.000

Data were expressed as the mean ± SD or number (%). For the data without homogeneity of variance and without a normal distribution, the nonparametric Kruskal-Wallis test was used. ACR, urinary albumin-creatinine ratio; eGFR, estimated glomerular filtration rate.

2.7 Diagnostic efficacy of serum sCD93, Cr and NGAL alone and in various combinations

The laboratory results for each indicator were exployed for ROC curve analysis (Table 8, Fig. 2), evaluating the diagnostic value of each indicator individually. For combinations of indicators, a binary logistic regression equation was established, and the predicted probability was used for the ROC curve analysis to evaluate the diagnostic value. The diagnostic cutoff value was the laboratory result or prediction probability corresponding to the maximum Youden Index (YI); this value indicates the maximum diagnostic performance for the observed indicator (YI = sensitivity + specificity − 1).

Table 8
Diagnostic performance of serum sCD93, Cr and NGAL

Item	AUC value	Cutoff value	Sensitivity	Specificity	YI	*P*
sCD93	0.750	301.707	0.542	1.000	0.542	0.000
Cr	0.899	67.500	0.804	0.957	0.760	0.000
NGAL	0.809	207.450	0.738	0.87	0.608	0.000
CD93 + Cr	0.905		0.776	1.000	0.776	0.000
Cr + NGAL	0.920		0.879	0.87	0.748	0.000
NGAL + sCD93	0.875		0.822	0.917	0.735	0.000
NGAL + sCD93 + Cr	0.942		0.879	0.913	0.792	0.000

When serum sCD93, NGAL and Cr were used individually, the AUC values were as follows, in increasing order: sCD93 (0.750), NGAL (0.809), and Cr (0.899). For the combination of 2 indicators, the AUC values were as follows, in increasing order: sCD93 + NGAL (0.875), sCD93 + Cr (0.905), and Cr + NGAL (0.920). Compared with detection using 1 indicator alone and 2 indicators combined, the diagnostic performance of 3 indicators combined was the highest (0.942).

3. Discussion

In this study, the correlation between serum sCD93 concentration and the prevalence of DN was explored, and the results had shown that serum sCD93 concentration was negatively correlated with eGFR and positively correlated with ACR. Furthermore, the risk assessment of renal complications indicated that the H-sCD93 group had a higher risk of DN compared with the L-sCD93 group. The reason could be attributed to the infiltration of inflammatory cells and the release of inflammatory cytokines in DM patients, which was an important pathogenesis mechanism of DN[18]. In fact, CD93 participated in the multistage inflammatory cascade and played an essential role in maintaining the plasma cell production of antibodies. Previous studies had shown that sCD93 was produced under inflammatory conditions and could in turn regulate the inflammatory function of monocytes and macrophages, inducing the differentiation of monocytes into macrophage-like cells. Then, these cells exhibited cell adhesion activation and enhanced phagocytic activity, thereby producing more reactive oxygen species, inflammatory cytokines and profibrotic cytokines, which leaded to a series of related complications[19].

Considering ACR was the first detectable change in DN[20], this investigation did the first to explore the relationship between serum sCD93 concentration and the level of microalbuminuria in T2DM patients. The results indicated that the serum sCD93 concentration in T2DM patients was significantly higher than that in the control group, and the serum sCD93 concentration in the L-ACR group was significantly higher than that in the N-ACR and M-ACR groups. The reason might be that sCD93 induced the differentiation of monocytes into macrophage-like cells, enhancing the stimulation of Toll-like receptors and producing proinflammatory cytokines, in particular, transforming growth factor β1 (TGF-β1, increased expression). TGF-β1 played an important role in inflammation and fibrosis together with connective tissue growth factor, platelet-derived growth factor and fibroblast growth factor-2. In addition, sCD93 was also involved in the activation of fibroblasts, leading to increased deposition of extracellular matrix in the interstitium and thicken of the glomerular basement membrane, which might promote podocyte apoptosis and increase glomerular vascular permeability. The above analysis indicated that sCD93 might play an important role in the development of DN[21]. In a word, exploration of the relationship between serum sCD93 concentration and the degree of microalbuminuria provided further evidence of the relationship between sCD93 and the progression of DN, indicating that sCD93 had potential value for monitoring the severity of DN for early intervention and corresponding treatments.

Cr was a traditional clinical indicator for evaluating renal function recommended by Kidney Disease: Improving Global Outcomes (KDIGO). NGAL was a newly discovered small molecular weight secreted protein that was highly expressed in various pathological tissues and cells with abnormal metabolism. The current studies had confirmed that the serum NGAL concentration increased rapidly during acute kidney injury[22]. This study had demonstrated that sCD93 was associated with DN and could reflect DN progression. Based on the analysis of the differences in renal function indicators between the DM and DN groups, the diagnostic efficacy of serum sCD93, NGAL, and Cr alone and in various combinations was further evaluated. The serum sCD93, NGAL, and Cr concentrations in the DN group were higher than those in the DM group, indicating the possibility of using combinations of the 3 indicators as a new diagnostic method for DN. The ROC curve analysis indicated that the serum Cr + NGAL + sCD93 triple test had the highest diagnostic efficiency (AUC = 0.942). On the basis of above data, the combination of 3 indicators provides a new biological means for the diagnosis and treatment of DN.

In summary, this study demonstrated that there was a good correlation between serum sCD93 and traditional markers of DN (eGFR and ACR), and sCD93 could reflect disease progression. Therefore, the inclusion of serum sCD93 as a supplementary diagnostic indicator for DN could improve the diagnostic efficacy for DN and played a role in guiding diagnosis, treatment and early intervention. However, this study had the following limitations. (1) The number of study subjects needed further increase. (2) Only the role of serum sCD93 concentration in DN was explored, but it might also play a role in other CKD models. (3) The eGFR and ACR were used to define DN, but these criteria also had their own limitations in the detection of DN. Therefore, more multicenter, large-sample studies were needed to further reveal the clinical application value of sCD93.

Declarations

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Notes

The authors have declared that no conflicting interests exist.

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Figures

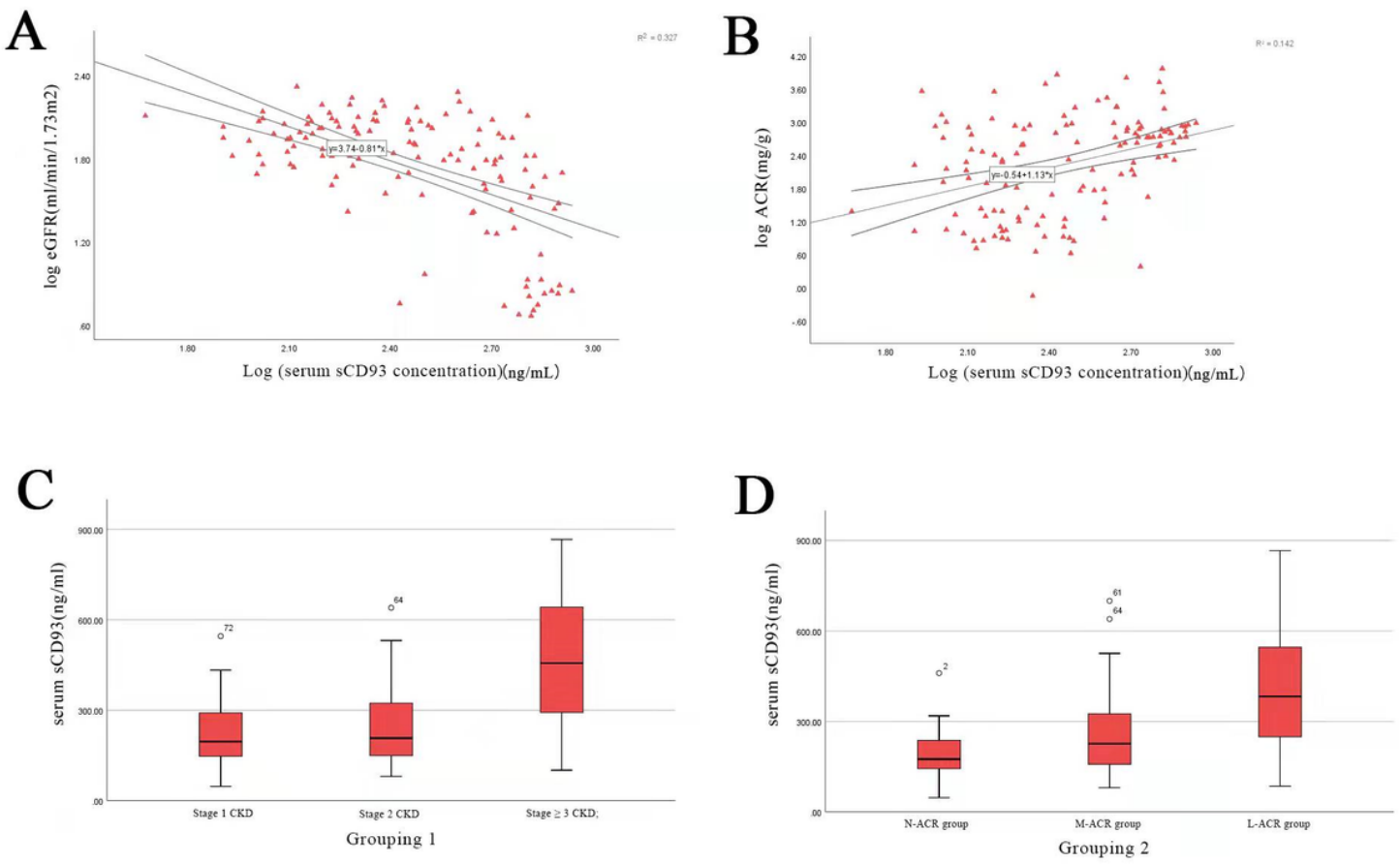

Figure 1

The relationships between serum sCD93 and clinical markers of DN. The correlations were analyzed by using the logarithmic transformation method and expressed by using the Pearson correlation coefficient (r) between serum

sCD93 and eGFR (Figure 1A), serum sCD93 and ACR (Figure 1B). The correlations were. The sCD93 concentration was showed with respect to the CKD stage (Figure 1C). The sCD93 concentration was showed with respect to the microalbuminuria stage (Figure 1D).

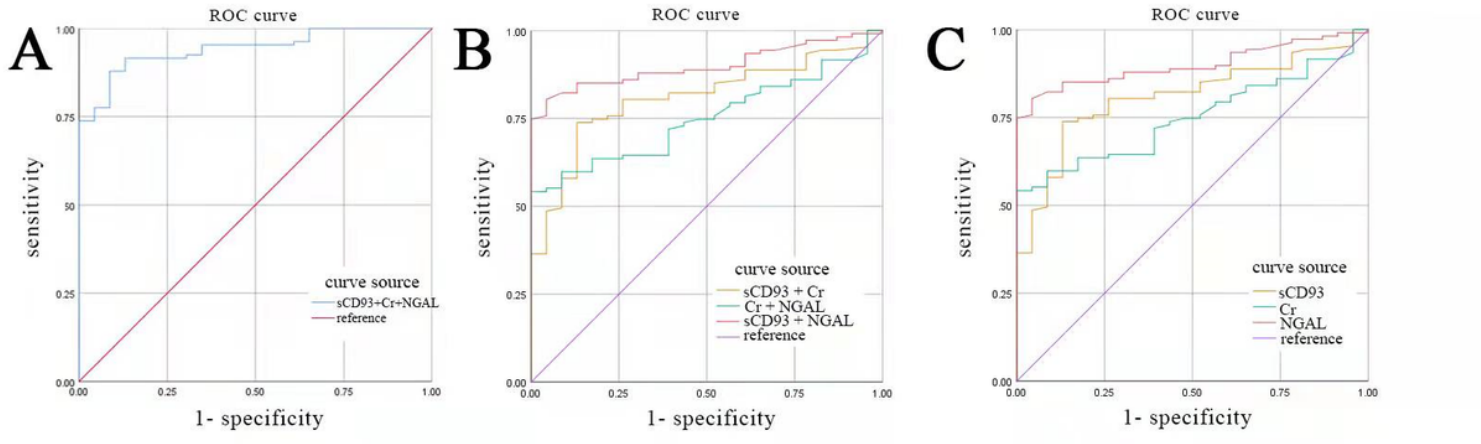

Figure 2

ROC curves for 3 indicators combined (Figure 2A), 2 indicators combined (Figure 2B) and each indicator (Figure 2C)

Supplementary Files

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